THE RELATION OF SOME RUSTS TO THE PHYSIOLOGY OF THEIR HOSTS

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E. B. MAINS

A DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in the
University of Michigan

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THE RELATION OF SOME RUSTS TO THE PHYSIOLOGY OF THEIR HOSTS[1]

E. B. Mains

I. INTRODUCTION

The relation of the rusts to their hosts has long occupied the attention of many workers, not only because of their economic importance, but more especially by reason of the extremely interesting biological problems which they offer. Not only have the rusts afforded a wide field for the study of the questions of immunity, susceptibility, physiological varieties, heteroecism, etc., but they, together with a few other groups such as the Peronosporales and Erysibaceae, make up part of the group of fungi which de Bary has called obligate parasites. This group of fungi is characterized by the requirement of a living host as the source of food supply. Saprophytes, on the other hand, obtain their food from dead organic material. Between the two classes are the intergrading facultative parasites and facultative saprophytes, determined by the degree a fungus is independent or dependent upon a living host. The saprophytic and facultative parasitic fungi have long been studied with attention to their food relations, but most of the work upon the obligate parasites has been confined to other lines, since the parasitic condition itself puts great difficulties in the way of an investigation of the nutrition of the fungus.

One must not overlook the fact that there are two conditions in obligate parasitism. In the first, we have the problems concerned with immunity and susceptibility, a condition which is common to all parasites whether obligative or facultative as well as to the facultative saprophytes. The other condition is that which goes to produce the

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obligative relation and make it seemingly impossible for the fungus to grow elsewhere than upon its host or hosts. Much work has been done and a number of theories developed with reference to the first condition; but concerning the second only a little work has been attempted, and but few theories advanced. Some authors in an endeavor to explain such parasitism have expressed the idea that the solution of the problem might be sought in the requirement of the fungus for some special nutrient which only its own particular host would be able to supply. What this nutrient might be, if such is the case, would be of extreme importance. Failing to determine this, it would be of not much less importance that some idea of its nature be obtained. Since the obligate parasites are distinguished by the absolute need of a living host for their food supply, it is from the host that the evidence for the solution of such a problem must be sought, and it is through the control of the various physiological activities of the host that one may hope to do this. It was to this end that this work was undertaken with the object of obtaining more data regarding the factors which control the obligate condition and determining, if possible, the substances or class of substances which are necessary for parasitism of this kind.

The work was carried on in the Cryptogamic Laboratory of the University of Michigan during the years 1914, 1915, and 1916 at the suggestion and under the direction of Dr. C. H. Kauffman to whom I am under deep obligations for many helpful suggestions and stimulating criticism.

II. HISTORICAL

The early history of the parasitism of the rusts has been well summarized by de Bary (1853), who was the first to study the rusts and smuts with scientific accuracy. According to de Bary, early naturalists such as Pliny, Theophrastus, Malpighi, Duhamel, Tillet, Tessier, and Plenk considered rusts not as the cause but as the result of a diseased condition brought about by atmospheric conditions. In the course of time, they were looked upon as foreign material which was partly the cause and partly the result of the disease. Later, the rusts were recognized as fungi by Linnaeus and Persoon, but they were still considered as the product of a diseased condition due to an injury such as the sting of an insect, etc. Unger (1834) believed that the rusts were produced by disarrangements in the respiratory organs of the plant due to which sap exuded into the intercellular spaces and there coagu-

lated, thus forming the rust. The next great step in the direction of a true understanding of the nature of the rusts was the recognition that they were the cause of the disease. The believers in this theory first concerned themselves with the study of the rusts as fungi and their entrance into their hosts. Léveillé (1839) showed that the rusts did not differ from saprophytic fungi in their development of mycelium and fruiting bodies except that they were within the living host. Prevost, according to the Tulasne brothers (1847), first observed the germination of rust spores. The Tulasne brothers and de Bary (1853, 1863) showed that the germ-tubes of the rusts enter through the stomata of their hosts and in some cases (the germ tubes of basidiospores) through the cell wall.

It was de Bary (1853), however, who finally definitely established that the rusts were parasites and that they were the cause and not the result of the disease. He concluded that the "Brandpilze," in which group he included both smuts and rusts, are to be considered as parasitic growths, since they arise from spores whose germ-tubes penetrate the host, develop a mycelium within the host's tissue, form spores, and finally break through the epidermis and infect other plants. De Bary in Die Brandpilze (1853, p. 109) defined a parasite as, "solche Pflanzen oder Thiere, welche auf lebenden Geschöpfen existiren, und ohne diese nicht bestehen können, welche durch den Reiz, den sie verursachen, durch die Nahrung, die sie dem Wohnorganismus entziehen, Störungen in dessen Organfunctionen hervorrufen; diese schwinden, sobald der Parasit entfernt oder getödtet wird." In view of this definition, de Bary's work on the "Brandpilze" was hardly sufficient to establish the rusts as parasites, since he did not show that they could not exist outside of living organisms.

A rather exhaustive search of the literature of this period does not reveal that any attempts were made to grow the rusts saprophytically. The general opinion which is now held appears to have arisen from the earlier idea that the rusts were diseased products of the host, first non-living and finally living products. In part this assumption of the obligate character of the rusts is due to the fact that they, unlike the facultative parasites, are never found in nature growing on other than living plants.

Among the later workers upon the obligate parasitism of the rusts is Brefeld (1883, 1908), who believes that the growing of rusts saprophytically is merely a matter of technique. He was able to obtain

secondary and tertiary spores from the basidiospores of some rusts, but further development was prevented by contaminations present in his cultures. Carleton (1903) used different media and a substratum as nearly like a wheat leaf as possible and obtained only a little difference in the length of the germ-tubes. Carleton, however, does not give an account of his methods. Ray (1901, 1903) has claimed to have cultivated a number of rusts upon decoctions of the host and on sterilized carrot and reports that in one case teleutospores were formed from the mycelium which was produced. Ray, however, gives only a very imperfect account of his methods. This coupled with the fact that he does not give either the species of rust or the kind of spores used subjects his results to criticism.

The germination of rust spores has received considerable attention especially from later workers. Plowright (1889), Eriksson and Henning (1894), Ward (1902*b*, 1903), Melhus (1912), Johnson (1912) and myself (1915) have found that temperatures between 10° and 30° C. are necessary for good germination and that the optimum temperature is between 12 and 18° C. Fromme (1913) has shown that a saturated atmosphere is necessary for abundant infection.

The factors controlling the inoculation of the host after the germination of the rust's spore have been principally investigated by Ward and his students. Miss Gibson (Ward, 1905) found that the germ-tubes of a number of rusts are able to penetrate into the intercellular spaces of plants other than their host without infecting. Furthermore Ward (1905) noticed this in the case of immune varieties of plants. He (1902*b*) also observed that the germ-tubes of *Puccinia dispersa* had a tendency to be negatively heliotropic and suggested that this may be a factor aiding in inoculation. Robinson (1914) in the case of *Puccinia Malvacearum* and Fromme (1915) and myself (1915) in the case of *Puccinia coronata* have shown a similar reaction of the germ-tubes. Balls (1905) believes that inoculation is brought about by a hydrotropic stimulus which causes the germ-tube to enter the stoma of its host.

The relation of the rusts to their host after infection has occupied the attention of a number of workers. De Bary (1887) and Jost (1907) have expressed the opinion that the predisposition of certain hosts for certain parasites is to be sought in the nature of the food which that host offers to them. De Bary (1887) and Tubeuf (1897) both have remarked that the rusts appear to adapt themselves to their host cells,

causing but little injury, at least up to the time of spore formation. Tubeuf, in the case of hypertrophies caused by rusts, thought that there appeared to be "a closer symbiotic relation between the fungus and its host branch than between the host branch and its main branch." He also noticed that in the case of some rusts the infected portion of the leaf remained alive after the death of the surrounding tissues and he looked upon this as a condition resembling that of some lichens. Ward (1890, 1902*c*) suggested that the relation between *Puccinia dispersa* and its host tends towards symbiosis and that the rust is not destroying the protoplasm of the host, but is robbing the host of its food supplies. Ward (1905) found that when the rust does attack the host so vigorously that the protoplasm is killed it brings about its own death and immunity for the host is produced. This condition, he discovered, can be duplicated by starving the host and by so doing starving the rust. His earlier work (1902*a*, 1902*c*) had already pointed towards this conclusion since he found that immunity did not depend upon anatomical features such as number and size of the stomata, hairiness, etc., and that mineral starvation, although it did not produce immunity, reduced the number of spores which were produced.

The relation of the rusts to the carbohydrate supply has been noticed by a number of workers. Halsted (1898) found in the case of *Puccinia Podophylli* that there is a collection of starch in infected regions of the leaf. The centers of such areas however contained much less starch than the margins. Robinson (1913) noticed much less starch in those areas of the leaf which are infected with *Puccinia Malvacearum*. McAlpine (1906) quotes the work of F. T. Shutt, who found that the grain and straw of rusted wheat contained more protein and less carbohydrates than the rust-free plants. Tischler (1912), working with *Uromyces Pisi*, discovered that the portion of the host containing the most mycelium of the rust also contained the greatest amount of sugar.

The effect of environmental factors such as soil, moisture, temperature, and light upon the relation of the rusts to their hosts has been studied by a number of workers. McAlpine (1906) noticed that nitrogenous manures retarded the ripening of grain, while phosphate of lime brought about early maturity and enabled the grain to escape the rust to some extent. Sheldon (1905) has reported that soils favorable to the host are also favorable to the rust of carnations (*Uromyces Caryophyllinus*).

Considerable difference of opinion has arisen concerning the effect of moisture on the development of the rusts. De Bary (1863) found that vegetative development of *Uromyces appendiculatus* and its production of spores was greatly increased by a humid atmosphere. McAlpine (1906) reported that drainage increased the yields of wheat, but did not decrease the rust. He also considered that irrigation late in the season tended to make the grain soft and brought on the rust. Stone and Smith (1899) and Blaringhem (1912) claimed that the rusts were favored by dryness. Sirrine (1900) and Buchet (1913), on the other hand, claimed that the rusts were favored by wet soils. Smith (1904) considered that a dry atmosphere retarded the development of the rust within the host while a dry soil favored development. Fromme (1913) found that for *Puccinia coronata* after infection has taken place moisture appears to have no effect upon the length of the incubation period.

The effect of temperature upon the development of rusts in their hosts has been but little studied. Sheldon (1902) found that the incubation period of *Puccinia Asparagi* was longer at an average temperature of 69° F. during the winter months than at an average temperature of 76° F. during the summer. It is likely that these results were in part due to the difference in the amount of light present in the two seasons. Fromme (1913) discovered that for *Puccinia coronata* a temperature between 20° and 30° C. brings about a shorter incubation period than a temperature of 14° to 21° C.

Fromme (1913) appears to be the only one who has definitely investigated the effect of light upon the development of the rusts. He found that when oats inoculated with *Puccinia coronata* were placed in darkness for a few days and then returned to the light, the incubation period was lengthened approximately by the time that the oats were in the dark. Fromme states that this may mean a dependence of the rust upon the transition products of photosynthesis and that this may explain the obligate parasitism of the rusts.

III. EXPERIMENTAL WORK

MATERIAL

Two rusts were employed, *Puccinia coronata* Cda. and *Puccinia Sorghi* Schw. *Puccinia Sorghi* was used in all of the experiments since its host maize (*Zea Mays* L.) was easier to work with. *Puccinia*

coronata was employed wherever oats (*Avena sativa* L.) could be used advantageously, and whenever time allowed. These two rusts were kept going on their host plants and thus stock material was always on hand. The method used was to make a spore suspension of the uredospores and spray them on the host by means of an atomizer. The pots were then covered with a belljar and placed where the temperature ranged between 14° and 25° C., a temperature of 20° C. being used whenever obtainable. The belljars were removed after 24 hours. When maize was used it was found to be advantageous to draw the leaves gently between the fingers before inoculating as the leaves are covered with a wax-like substance which causes the spore suspension to roll off without wetting them. In some experiments, definite areas were inoculated by placing spores on them with a spear-pointed needle after the plant had been atomized. During the winter, the cultures were kept in a greenhouse, where *Puccinia coronata* required renewal about every three to five weeks and *Puccinia Sorghi* about every two months. This was done by inoculating freshly grown plants. During the summer, the stock cultures were kept in a garden in the open, where the rusts propagated themselves.

Development of the Rusts

Puccinia coronata

The first signs of infection show in from five to seven days after inoculation, when light green areas are formed on the leaves. About seven to eleven days after inoculation, pustules appear in these areas as small, yellowish swellings, which soon break through the epidermis liberating the mass of uredospores. Teleutospores develop in about twenty-nine to thirty-six days after inoculation, when uredospore production has about ceased and the leaf is slowly dying and drying up. They show as blackish zones usually at the margins of the infected areas and their first appearance occurs towards the apex of the leaf, which is also the part of the leaf which first begins to die.

Puccinia Sorghi

The first signs of infection on maize are also light-colored areas on the leaves. These appear in about six to seven days after inoculation and pustules develop soon afterward, in usually seven to ten days.

The pustules make their appearance first on the lower side of the leaf and are more abundant and larger there, often becoming confluent.

The development of the rust within the host can be followed by sectioning day by day after inoculation. The method employed, which gave very good results, consisted in sectioning the infected leaf between pith. Very thin sections can be obtained in this way, if the leaf is cut up and a number of thicknesses of the leaf are placed between pieces of pith. The sections obtained in this way were mounted in Stevens's chloral hydrate and iodine (Stevens, 1911). The sections are cleared by this mixture so that the hyphae of the rust stand out, showing clearly the haustoria in the cells of the host. Chlorophyll, which in untreated sections interferes with the determination of the mycelium, is thus bleached out, and any starch which may be present can be recognized. At the end of the third day, mycelium was already found to be well developed. At this time, the amount of starch, which was normally present in the parenchyma sheaths of the vascular bundles, was only slight, or it was entirely absent. By the fourth day, the mycelium had formed dense masses in the intercellular spaces of the mesophyll of the leaf. None was found sending haustoria into the epidermal cells, nor was any mycelium found in the vascular bundle and its parenchyma sheath. The cells of the parenchyma sheaths in the infected areas did not show at this time so large a quantity of starch as those in the non-infected areas. By the fifth day, the epidermal cells were invaded by haustoria from neighboring hyphae, and the amount of starch was noticeably less in the parenchyma sheaths of the infected areas. About the sixth day, pustules appeared. These were formed from masses of mycelium just below the epidermis. No mycelium was found in the vascular bundles or their parenchyma sheaths at this time. Some starch was found in the parenchyma sheaths of the infected areas, but these did not stain so darkly with iodine as those of the non-infected areas.

The development of the rust progresses by a continued spread of its mycelium and the production of more pustules. The infected areas are however always limited in extent, varying from a millimeter to about a centimeter in diameter. Often, in the case of heavy infection, whole leaves may become covered with pustules due to the union of many infected areas. Mycelium is found throughout the intercellular spaces of the infected areas, where it sends its hyphae into the neighboring cells, forming the branched, finger-like haustoria, which

have been well illustrated by Evans (1907). The vascular bundles are apparently never invaded by the mycelium and the parenchyma sheaths which surround them only occasionally have haustoria in their cells.

When limited areas of the leaf are infected, an interesting phenomenon takes place. After the tissue has been infected for some time—in some cases in so short a time as nine days—the ends of the leaf beyond the infected areas begin to die and the regions immediately surrounding become yellowish, while the infected areas retain the green color of healthy tissue. The infected areas, of which there may be two or three on the same leaf, often become surrounded by dead tissue, except perhaps for the midrib itself. The infected areas themselves still retain their original green color, and sections show that the cells of these regions have all the appearance of normal cells, except for the presence of haustoria within them. They are turgid and filled with green chloroplasts. The neighboring tissue, on the other hand, is brown and the cells are shrivelled up and dead.

Effect of Temperature upon Development

Puccinia coronata

Experiments 1 and 2.—Two experiments were carried out to discover the effect of temperature upon the development of *Puccinia coronata* within its host. In each, six pots of oats were used. These were inoculated by spraying with a spore suspension of the uredospores, and, after being left for 24 hours under belljars, four pots of each set were removed to a well-lighted room where the temperature averaged 15° and 13° C. respectively for the two experiments. Two from each set were kept in a similar room where the temperature averaged 20° C. The incubation period of the rust at 20° C. in both experiments was 9 days. The incubation period of the rust at 15° C. in the first experiment was 13 to 15 days, and the incubation period for the rust at 13° C. in the second experiment was 15 days.

The results of these two experiments thus indicate that low temperatures retard the development of the rust in its host.

Puccinia Sorghi

Experiment 3.—This experiment was carried out with *Puccinia Sorghi* on corn in the same manner as were the two preceding experi-

ments. Three pots of corn were kept at a temperature averaging 20° C. and three at a temperature averaging 13° C. The incubation period of the rust at 20° C. was 7 days while that at 13° C. had an incubation period of 13 days, showing that low temperatures retard the development of *Puccinia Sorghi* in its host.

Experiment 4.—Six pots of corn were inoculated with uredospores of *Puccinia Sorghi* and kept under a belljar at a temperature of 18° C. for 12 hours. Two of the pots were placed under belljars in an Eberbach electric incubator at 40° C. The outer door of the incubator was left open, allowing light to enter, and the incubator was placed in an east window. In a similar manner two pots were kept in an incubator at 30° C. Even with the outer doors open these incubators maintained a temperature varying only a few degrees. The other two pots of corn were placed under belljars in a box about the size of the incubators with the open side facing the window. These two pots were at room temperature, which according to a thermograph averaged 18° C. The belljars were removed once a day from all the plants in order to renew the oxygen supply.

At the end of the fourth day, the two pots of corn at 40° C. were dead. Pustules appeared on the plants at 18° C. in seven days. The only sign of rust on those at 30° C. at this time was the greenish spots mentioned before as remaining in infected areas when the rest of the leaf is yellowing. Sections through these areas showed a mycelium which was only sparingly developed. At the end of fourteen days, no pustules had formed on the plants at 30° C. At this time, most of the leaves were dead, only the upper still retaining a green appearance.

These results show that a temperature of 30° C. or higher prevents the development of *Puccinia Sorghi* in its host.

Effect of Humidity upon Development

The work, as far as carried out, was done with *Puccinia Sorghi.*

Experiment 5.—Fourteen plants of maize were inoculated with the uredospores of *Puccinia Sorghi* and kept under belljars at 18° C. for twelve hours. Four plants were then placed in a south window under belljars, by which means they were kept in a nearly saturated atmosphere. The remaining ten plants were placed without belljars on the table beside the other four. The earth in the pots of five of these ten

was kept saturated with water. The other five were watered just enough to prevent the plants from wilting. The humidity of the room varied between 20 and 36 percent and the temperature averaged 24° C. At the end of nine days after inoculation the number of pustules on the plants was counted.

The plants in dry air and moist soil averaged 51 pustules per plant. These pustules, however, were small and not as well developed as in the others. Plants in a dry atmosphere and wet soil averaged 151 pustules per plant. These pustules were large and well filled with spores. Plants in a saturated atmospheric and wet soil averaged 371 pustules per plant. These pustules were large and difficult to count as many of them had become confluent.

At the end of 25 days, the infected leaves of plants in dry air and moist soil were all dead and dried up, and the new leaves were free from the rust. The plants in dry air and wet soil had a few pustules on a few old live leaves, but most of the infected leaves were dead. The new leaves upon these plants were free from the rust. Upon the dry, dead leaves of these plants green areas surrounding the pustules were still evident. The tissue surrounding these areas had the brown appearance of tissue whose cells had disintegrated before they had dried. This would indicate that the green areas surrounding the pustules had died because of the drying out rather than because of the effect of the rust. The plants in wet soil and saturated atmosphere still had a number of live leaves heavily infected with rust. Some of the leaves however were dead or dying. On the latter, the infected areas remained green surrounded by yellow, sickly tissue or by brown tissue composed of dead cells. The new leaves had a small number of pustules showing that by this time some reinfection had taken place.

The development of *Puccinia Sorghi*, as shown by the number of pustules produced, is thus favored by the saturated atmosphere on the one hand and by the wet soil on the other. The length of the incubation period, however, is not much influenced. In dry air, the plants finally become free from the rust by the drying up of the infected leaves; and reinfection does not take place since spore germination is prevented in dry air. In a saturated atmosphere, the infected leaves live for a longer time since they do not dry up; and reinfection also takes place to some extent, since the spores produced are able to germinate in the humid atmosphere.

Effect of Mineral Salts upon Development

Two experiments were carried out to discover the effect of mineral salts upon the development of *Puccinia Sorghi*. In the first, water cultures were used, while in the second pure quartz sand watered with the solutions was employed.

Experiments 6 and 7.—The solutions used in the first of the two were Knop's full mineral nutrient solution, as given by Jost (1907) and Miss Wuist (1913), and solutions in which one of each of the eight principal elements (Mg, Ca, K, Fe, S, N, P, and Cl) were lacking. The full nutrient solution consisted of the following in 1,000 cc. of distilled water:

$MgSO_4$	.25 g.
$Ca(NO_3)_2$	1.00 g.
KH_2PO_4	.25 g.
KCl	.12 g.
$FeCl_3$	trace.

The nutrient solution minus calcium was made by substituting KNO_3 for $Ca(NO_3)_2$. K_2SO_4 was substituted for $MgSO_4$ to form a solution minus Mg. $Ca(H_2PO_4)_2$ and $MgCl_2$ were substituted for KH_2PO_4 and KCl to form a solution minus K. $MgCl_2$ was substituted for $MgSO_4$ to form a solution minus S. $Ca(H_2PO_4)_2$ was used in place of $Ca(NO_3)_2$ to give a solution minus N. KNO_3 was used in place of KH_2PO_4 to give a solution minus P. KNO_3 and $FePO_4$ were used in place of KCl and $FeCl_3$ to give a solution minus Cl. And $FeCl_3$ was left out of the full solution to form a solution minus Fe.

In the second experiment, maize was planted in quartz sand which had been thoroughly washed with distilled water and then dried. Knop's full mineral nutrient solution of three times the ordinary strength was used. The other nutrient solutions were made up a little different than in the preceding experiment in that, when an element was omitted, the concentration of the other elements in the solution was maintained except for the element it was replaced by. Thus for example, in $-$Mg solutions .75 gm. of $MgSO_4$ was replaced by 1.04 gm. K_2SO_4 so that there was as much SO_4 present as before. The amount of the replacing element, K, naturally increases. In the $-$Ca solution, $Ca(NO_3)_2$ was replaced by $Mg(NO_3)_2$ and KNO_3 so that there would not be too great a preponderance of either Mg or K in the solution.

Kernels of corn were planted in battery jars containing 1.2 kg. of

quartz sand prepared as above and the jars were then placed in the greenhouse. The various solutions were used to water the plants. After 35 days, the plants were inoculated and placed in a moist chamber at 20° C. for twelve hours.

In both experiments sections through the infected areas showed that from about .5–1.2 mm. on each side of the pustules, there was no starch in the parenchyma sheaths, although it was present in considerable quantities in the rest of the leaf. Beyond this for about .2–.6 mm. on each side, the concentration of the starch gradually increased until it reached the full concentration of the rest of the leaf.

TABLE I

Effect of Mineral Starvation upon the Development of Puccinia Sorghi

Solution	Condition of Plants		Number of Plants Used		Number of Plants Infected		Average No. Pustules per Plant
	Water Culture	Sand Culture	Water Culture	Sand Culture	Water Culture	Sand Culture	Sand Culture
Full solution..	green	green	2	5	2	4	197
— Ca.....	yellowish	dead	3	5	1	0	0
— Fe.....	"	light green	4	5	4	4	31
— Cl.....	"	" "	4	5	4	3	170
— Mg.....	"	" "	4	5	4	2	8
— K.....	green	" "	4	5	3	3	32
— P.....	dark green	" "	4	5	4	2	12
— N.....	light "	" "	3	5	3	3	11
— S.....	green	" "	4	5	3	2	13

The results as given in Table I show that mineral starvation does not prevent infection of *Puccinia Sorghi* but only that the amount of rust as shown by the number of pustules is less. Starch is prevented from forming in the immediate vicinity of the pustules.

EFFECT OF LIGHT UPON DEVELOPMENT

The effect of light upon development of the rusts was studied in a set of experiments, the results of which are given below. A number of pots of the plants were inoculated under the same conditions. Some were then placed under belljars in the light, and the rest were covered with dark cylinders. After a few days, the plants under the dark cylinders were placed in the light and the incubation periods of each recorded.

Puccinia Coronata

Experiments 8, 9, and 10.—In these experiments inoculation was accomplished as stated above and the results obtained are given in the following table.

TABLE II

Effect of Light upon Development of Puccinia Coronata

Experiment	Pot No.	Time in Dark	Time in Light	Incubation	Retardation
	C 17.1		10 days	10 days	
	C 17.2		10 "	10 "	
	C 17.3	5 days	10 "	15 "	5 days
8	C 17.4	5 "	10 "	15 "	5 "
	C 17.5	18 "		died, no infection	
	C 17.6	18 "		died, no infection	
	C 17.7		10 "	7–10 days	
	C 17.8		10 "	7–10 "	
	C 17.9	7 "	6 "	12–13 "	6–7 "
9	C 17.10	7 "	6 "	12–13 "	6–7 "
	C 17.11	20 "		no infection	
	C 17.12	20 "		" "	
	C 17.13		10 "	10 days	
	C 17.14		11 "	11 "	
	C 17.15	7 "	5 "	13 "	2–3 "
10	C 17.16	7 "	5 "	13 "	2–3 "
	C 17.17	20 "		no infection	
	C 17.18	20 "		" "	

The results of these experiments show that in the absence of light the development of *Puccinia coronata* is retarded and if left in darkness too long, the rust is killed.

Experiment 11.—In the preceding experiments, the plants were all placed in a dark moist chamber after spraying with the spore suspension. Fromme (1915) and I (1915) have found that the germ-tubes of the uredospores of this rust are negatively heliotropic. It seems possible from this that the retardation of the appearance of pustules might have been due to a failure of the germ-tubes to enter the host while in the dark. Then, when brought into the light, inoculation might have taken place from spores whose germination had been delayed. To test this a fourth experiment was set up in which some of the plants, after being sprayed with the spore suspension, were left under belljars in the light for from one to five days and were then placed under dark cylinders for various periods, after which some of them were returned to the light. After spraying with the spores,

others were kept in the dark for various periods of time and were then brought into the light.

TABLE III

Effect of Light Upon the Development of Puccinia Coronata. Comparison of Plants Inoculated in Light and in Darkness

Pot No.	Time in Light	Time in Dark	2d Period Time in Light	Incubation Period	Retardation
C 17.19.....	1 day	4 days	6 days	11 days	2 days
C 17.20.....	1 "	4 "	6 "	11 "	2 "
C 17.21.....	1 "	19+ "		no infection	
C 17.22.....	5 "	15+ "		" "	
C 17.23.....	5 "	6 "	3 "	14 days	5 "
C 17.24.....	5 "	6 "	3 "	14 "	5 "
C 17.25.....	9 "			9 "	
C 17.26.....	9 "			9 "	
C 17.27.....	9 "			9 "	
C 17.28.....		5 "	7 "	12 "	3 "
C 17.29.....		1 "	8 "	9 "	
C 17.30.....		1 "	8 "	9 "	
C 17.31.....		5 "	6 "	11 "	2 "
C 17.32.....		20+ "		no infection	
C 17.33.....		1 "	8 "	9 days	
C 17.34.....		5 "	7 "	12 "	3 "
C 17.35.....		11 "	10 "	21 "	12 "
C 17.36.....		5 "	7 "	12 "	3 "

Following this experiment, plants were inoculated in areas marked with India ink and covered with dark cylinders. After two days, the inoculated areas were sectioned and the sections treated with chloral hydrate and iodine. Mycelium was found in some of the inoculated areas, but had not developed to a very great extent.

From these results it is evident that infection of *Avena sativa* by *Puccinia coronata* takes place in darkness as well as in light, although apparently the amount of infection is less in darkness.

Puccinia Sorghi

The first experiments upon the effect of light upon the development of *Puccinia Sorghi* were carried out in the same manner as with *Puccinia coronata*. The results obtained in these first experiments (Experiments 12–19) were not as clear cut as those obtained with the latter. Seven out of eleven plants which were in the dark three to eight days before being placed in the light had their incubation period lengthened one to two days. The other four had no retardation of their incubation period Four out of thirteen plants which were in

the dark throughout the experiment became infected. Their incubation period was lengthened only two days. The remaining nine of the thirteen, however, remained uninfected.

Experiment 20.—In this experiment the procedure was the same as in the previous experiments, except that the plants were kept in the dark for five days to exhaust them as much as possible of carbohydrates. In this case, two out of the seven plants which were put in the dark for seven days did not have their incubation period lengthened at all. The other five had their incubation period lengthened from 2 to 4 days, which is shorter than the time they were in the dark. Infections occurred on two out of twelve of the plants in the dark for the entire time and in these cases the incubation period was lengthened. Since these results more nearly agree with those obtained with oats (*Avena sativa*), it would appear that the reserve food supply of the maize is to be considered as the cause of the disagreement. In the case of maize, the endosperm furnishes considerable food to the plant during the first month and by the time this is exhausted, the plant is of such a size that considerable reserve food is stored up in the stem and other organs of the plant. The next experiment was carried out with the object of exhausting this reserve as nearly as possible before inoculating.

Experiment 21.—In this experiment, young plants were used. In order to control the reserve food supply of the host as much as possible, maize was germinated in a moist chamber and after four days the endosperms were dissected away. The plants were then planted in quartz sand which had been moistened with Knop's solution and were left in the light until the leaves were out and the plants had taken on a green color. Following this they were removed to the dark for three days to exhaust the carbohydrates manufactured during this time. All were finally inoculated with uredospores of *Puccinia Sorghi* and four kept under belljars and eight under dark cylinders. Five of the latter were removed at various intervals and placed under belljars. Three were left under dark cylinders throughout the experiment.

From the results of this experiment, it is evident that when the reserve food supplies of the host are cut down to the minimum, the incubation period of the rust is lengthened to a period corresponding to the time that the host was placed in the dark and that when the host is kept in the dark, there is no development of the rust. Not only does this indicate a direct relation to the carbohydrate supply

TABLE IV

Effect of Light Upon Development of Puccinia Sorghi. Plants Exhausted as Nearly as Possible of Soluble Carbohydrates

Plant No.	Age of Plant	Endosperm	Time iu Dark	Time in Light	Incubation Period	Retard-ation
FC–E 50....	13 days	without		5 days (died)	no infection	
FC–E 51....	13 "	"		7 days	7 days	
FC–E 52....	13 "	"		7 "	7 "	
FC–E 55....	13 "	"		8 "	8 "	
FC–E 54....	13 "	"	3 days	7 "	10 "	3 days
FC–E 58....	13 "	"	3 "	6 "	9 "	2 "
FC–E 59....	13 "	"	3 "	7 "	10 "	3 "
FC–E 56....	13 "	"	6 "	7 " (died)	no infection	
FC–E 60....	13 "	"	6 "	6 days	12 days	5 "
FC–E 61....	13 "	"	6 " (died)		no infection	
FC–E 53....	13 "	"	10 days		" "	
FC–E 57....	13 "	"	13 "		" "	

of the host, but the apparent exceptions in the previous experiments due to the presence of reserve food in the host only strengthen this conclusion the more.

EFFECT OF THE LACK OF CARBON DIOXIDE UPON DEVELOPMENT

The preliminary experiments to show the effect of the lack of carbon dioxide upon development were not satisfactory, since the plants used possessed an endosperm and derived their food supply from it as was shown by the plants in a minus carbon dioxide atmosphere developing as well as those of the check. Infection occurred at the same time as in the checks or a few days later. Since the experiments with light, which were being run at the same time, pointed to carbohydrates as factors in the development of the rusts, it was evident that the plant must be deprived as nearly as possible of carbohydrates. This was attempted in two experiments.

Maize was germinated in a moist chamber at 30° C. and at the end of six days, when the plumule had reached the length of five to six centimeters, the endosperm was dissected away and the plants were planted in small bottles filled with quartz sand and moistened with Knop's solution. The plants were grown in the light for a few days until the leaves were expanded and chlorophyll had developed. They were then placed in a dark chamber for three days to exhaust them of

the carbohydrates which had been formed while in the light. Following this, the plants were inoculated with uredospores of *Puccinia Sorghi* and placed in large-mouthed liter bottles.

A carbon-dioxide-free atmosphere was obtained in these bottles by placing a strong solution of potassium hydroxide in the bottom of each and the oxygen supply was maintained by connecting the bottles with U tubes which contained a mixture of pumice-stone moistened with a KOH solution and pieces of KOH. Checks were run which were set up similarly, with the exception that KOH was omitted (Plate IV, figure 1). All the joints and corks were coated with paraffine.

Two experiments were conducted in this manner, the results of which are given below.

Experiment 22.—In the first experiment, plants treated as above were inoculated and six (FC–E 60–65) were placed in bottles having a carbon-dioxide-free atmosphere and two (FC–E 66 and 67) in check bottles. The set was kept in the dark for 24 hours to allow the apparatus to become free from carbon dioxide and then was placed in the light of an east window at an average temperature of 22° C. In four days, numbers FC–E 60–65 began to show the effects of the lack of carbon dioxide by their sickly appearance. Numbers FC–E 66 and 67 remained fresh and healthy. Pustules first showed on Numbers FC–E 66 and 67 in eight days. The plants in carbon-dioxide-free atmosphere at this time showed no signs of infection. Numbers FC–E 64 and 65 were dead and the upper parts of the leaves of Numbers FC–E 60–63 were also dead.

The experiment was finished on the eleventh day. At this time no infection had taken place upon any of the plants in the carbon-dioxide-free atmosphere.

Experiment 23.—Eight plants (FC–E 68–75) which were treated as in the preceding experiment were inoculated with spores and placed under a dark cylinder for 12 hours at 18° C. Six of these (FC–E 68–73) were then placed in bottles with a carbon-dioxide-free atmosphere and two (FC–E 74, 75) were used as checks. The bottles were all kept in the dark for 12 hours so that the plants might not manufacture carbohydrates while containing carbon dioxide. Pustules appeared on both the checks in six days and on the seventh day pustules appeared on FC–E 69 and 73 in small numbers. These two plants, however, appeared fully as healthy in every way as the checks, while FC–E

68, 70, 71, 72 plainly showed the lack of carbon dioxide in their lighter green leaves, which were dying back from the tip.

The experiment was finished at the end of the twelfth day. There was no infection on FC–E 68, 70, 71, 72, which, although they showed indications of approaching death, were still alive. Not even greenish spots showed on the yellowing leaves such as often appear upon dying leaves in infected areas. FC–E 69 and 73 at this time had a number of open pustules and the plants themselves were in every particular as healthy as the checks, showing that the apparatus in these cases was defective and did not eliminate entirely all the carbon dioxide, or that the plants possessed a large enough amount of food at the beginning of the experiment to supply them.

These two experiments show, as do those with light, that there is a relation between the development of the rust and the carbohydrate supply.

Effect of Supplied Carbohydrates upon Development

Since the preceding work indicated a relationship of the rust to the carbohydrate supply, further experiments were undertaken in order to study this relationship more thoroughly. To do this it is necessary to supply carbohydrates to the host which has been deprived of them as nearly as possible, since the experiments with light indicate that the host normally contains more or less carbohydrates in available form for the use of the rust.

This work divides itself into two parts according to the manner of supplying the carbohydrates to the host. In the first case, carbohydrates were supplied to the plant through the scutellum of the maize seedling and through the roots. Van Tieghem (1873) has shown that embryos can be developed upon starch. Brown and Morris (1890) have found that not only do excised embryos develop normally upon starch, but that they will also do so with sugar solutions, especially cane sugar, and that when both starch and sugar are present, the sugar is used up before the starch is attacked.

A number of authors have investigated the ability of plants to take up carbohydrates through their roots. Mazé and Perrier (1904) obtained a good growth of maize in 1 percent glucose and sucrose. Acton (1889) found that in this way acrolein, acrolein-ammonia, allyl alcohol, glucose, acetic aldehyde, ammonia, glycerine, laevulenic acid, calcium laevulinate, cane sugar, inulin, dextrins, glycogen, and extract

of natural humus can be used by a number of plants when in a carbon-dioxide-free atmosphere. J. Laurent (1897, 1898, 1904) investigated this subject thoroughly for corn. He found that glucose, invert sugar, sucrose, soluble starch, and starch can be taken up by corn through its roots and utilized in a carbon-dioxide-free atmosphere and to a somewhat less degree in a normal atmosphere in the dark.[2]

In the second case, cut pieces of leaf were floated upon various carbohydrate solutions. A number of workers have shown that portions of plants may utilize sugar from solutions in which they were placed. Boehm (1883) showed that cut pieces of leaf of *Phaseolus multiflorus* upon solutions of cane sugar and glucose form starch in the dark. E. Laurent (1886) has shown that etiolated potato sprouts placed in the dark with their cut ends in a 10 percent cane-sugar solution grow for more than five months and form starch. A. Meyer, according to Acton (1889), found that shoots, when supplied with dextrose, glycerine, sucrose, and inulin, can form starch. A number of other authors have carried out similar experiments with like results.

Carbohydrates Supplied to Seedlings

In order to grow plants in carbohydrate nutritive solutions, it is necessary to have both the plants and solutions as nearly sterile as possible or the solution will be quickly filled with the growth of a number of saprophytic fungi. The only feasible way to obtain sterile seedlings is to sterilize the seed. A number of methods have been used by different workers for sterilizing seeds, but almost all of these methods have been found inefficient in some particular by other workers.

Ward (1902*d*) used "various antiseptics" and also heated the seeds to 60–70° C. for the purpose of sterilizing brome seeds. These were then placed in sterile petri-dishes to germinate. Laurent (1897, 1904) used .2 percent $HgCl_2$ solution for 1½ to 3 hours for corn. J. K. Wilson (1915) has recently reported good results for a number of seeds from the use of a solution of chlorine obtained from bleaching powder. A number of other antiseptics have been used, such as H_2SO_4, $CuSO_4$, H_2O_2, phenol, HNO_3, etc., none of which have been found generally or uniformly successful. Of these H_2SO_4, $HgCl_2$, and the calcium hypochlorite method of Wilson were tried by myself.

[2] Since the above was written Kundson (1916) has also shown that maize is able to take up through its roots dextrose, laevulose, maltose, and sucrose from their 2 percent solutions with an increase in growth and dry weight.

In the use of the first, grains of yellow dent corn were cleaned and dropped into concentrated H_2SO_4 for ten minutes. They were then removed to a capsule of sterile distilled water and washed once or twice with sterile distilled water. They were then placed in sterile, moist chambers. This treatment with H_2SO_4 did not appear to injure the seed in any way. On the other hand, it hastened germination of the seed. But it was not effective in killing the particular fungus spores which were present on these occasions and in nearly every case, fungi developed in the moist chambers.

The use of $HgCl_2$ was found to be much more satisfactory and it has been used entirely in this work where sterile corn plants were needed. The method used was to clean the seeds and drop them into .5 percent $HgCl_2$ solution where they were left for thirty minutes. The $HgCl_2$ solution was then poured off and replaced several times with sterile distilled water. The corn was then removed with sterile forceps and placed in large (4.3 × 25 cm.) sterile test tubes, which were placed in an incubator at 27°–30° C. This method gave very good results throughout the work and only a small number of contaminated seedlings were found.

Wilson's calcium hypochlorite method was tried, but it did not give very good results. Ten grams of "Acme" chloride of lime (bleaching powder) containing 30 percent chlorine were mixed with 140 cc. of water according to Wilson's directions and maize was treated for 9 hours with the filtrate. Only very slight germination and poor seedlings were obtained by this treatment. The corn used however was a little over a year old and this may account for the failure, although it gave very good germination when treated with $HgCl_2$, as stated above. To test this method still further corn was taken out of the sterilizing solution every hour for eight hours and placed in sterile moist chambers and then placed in an incubator at 27° C. Good germination took place with corn treated for one and two hours, but when treated beyond that time the germination was poor. All the moist chambers contained more or less contaminated seedlings. This method when used with oats and wheat gave good results, while $HgCl_2$ as outlined above was unsatisfactory with oats and wheat.

Although the number of disinfecting solutions used was not very great, yet the results obtained point to the uselessness of trying to obtain a disinfectant which will work for all seeds under all conditions. Not only will the kind of seed but also the age of the seed, the amount of

water contained in it, the permeability of the seed coat, and the kind of fungus spores on it—all variable factors—have important bearing upon the effect of sterilizing solutions upon the seed. It is very unlikely that any one solution will work effectively under all combinations of these conditions. Such a situation means that each worker must select the agent which is the best suited to meet the requirements of his particular conditions.

The corn after having been sterilized with $HgCl_2$ was germinated in large sterile test tubes. The necessary moisture was maintained in the tube by having absorbent paper saturated with distilled water in the bottom of the tube when sterilized. The tubes were kept in the dark in incubators at a temperature of 27°–30° C. for about seven days, at which time the plumule of the corn had attained the height of 5–15 cm.

The endosperm of corn contains a quantity of nutriment which can nourish the plant for about a month and in fact when the plant is grown in the light, the endosperm often lasts much longer, even two months. It is therefore necessary to remove the endosperm before placing the plants in nutrient solutions, since it would furnish all the plants of the experiment with a large carbohydrate supply. This is done by removing the plant from the test tube with sterile forceps and making a longitudinal cut through the endosperm down to the scutellum. The action of diastase has by this time dissolved away the portion of the endosperm lying next to the scutellum and the two halves of the endosperm are easily removed with sterile forceps, leaving the scutellum surface exposed. The plants are then placed in their nutrient solutions.

The following solutions were used: Cane sugar 15, 12, 6, and 3 percent; cane sugar 10 and 3 percent plus Knop's mineral nutrient; cane sugar 10 percent plus Knop's mineral nutrient minus nitrogen (see Experiment 6); starch jelly 15 percent; starch jelly 15 percent plus Knop's mineral nutrient; starch jelly 15 percent plus Knop's mineral nutrient minus nitrogen; dextrose 3 percent; dextrose 3 percent plus Knop's mineral nutrient; maltose 3 percent; maltose 3 percent plus Knop's mineral nutrient; dextrin 3 percent; dextrin 3 percent plus Knop's mineral nutrient; Knop's mineral nutrient; Knop's mineral nutrient minus nitrogen; distilled water.

Erlenmeyer flasks of 150 cc. capacity were used to contain the solutions. These were stoppered with cotton plugs and autoclaved

at 110° C. for 30 minutes. It was found that there was much less contamination of cultures if the flasks were autoclaved just before using and allowed to cool in the autoclave. If this was not possible, the flasks were usually wiped off with a corrosive sublimate solution before using, since the roots of the corn plants often touched the outside of the flasks while they were being placed in the solution due to the small neck of the flask. The cotton plugs used to stopper the flasks were replaced after flaming and as they were made rather loose and somewhat larger than the necks of the flasks, they fitted rather closely around the corn stems (Plate IV, figure 2).

The flasks with plants thus prepared were placed in a moist dark chamber. This chamber was prepared by covering a galvanized tank ($1\frac{1}{4} \times 1 \times 3$ ft.) with a cover made of heavy black paper. The cover was a little larger than the tank and reached to the bottom on the sides, so that light was excluded and ventilation permitted. A layer of water was kept in the bottom of the tank while the work was being carried on in the greenhouse; this was enough to maintain a saturated atmosphere in the chamber. Later when the experiments were conducted in the drier air of the laboratory, coarse woven cloth which was kept wet was spread over the tank under the black paper lid in order to maintain the saturated atmosphere.

After the plants had been in this dark chamber for several days, they were inoculated by spraying with a spore suspension and in addition a small quantity of spores was placed on certain leaves. They were kept at 20° C. for 24 hours, at which temperature the uredospores of *Puccinia Sorghi* germinate vigorously.

The results of Experiments 24 to 31 are given in the following table. In all the cases where infection took place upon plants in Knop's nutrient solution or distilled water, the pustules were poorly developed and few in number. In the three experiments where such infection occurred, the infected portion of the leaves were cut off and the plants were reinoculated. No infection took place the second time on such plants in distilled water or Knop's nutrient. Plants in sugar solution, however, were infected, although to a somewhat less extent than in the previous experiments. The reinfected plants were left until all of them died. It was found that the plants which were infected in Knop's and distilled water in the original experiments lived as a rule longer than the others. This would indicate that infection in the original experiments upon these plants was due to a supply of food

TABLE V

Effect of Carbohydrate Supplied to the Seedling Upon the Development of Puccinia Sorghi

Solution	No. of Plants Used	No. of Plants Infected	Av. Growth per Plant per Day
Starch 15%	10	9	3.4 mm.
Starch 15% + Knop's	10	3	1.6 "
Starch 15% + Knop's − N	10	5	5.1 "
Cane sugar 15%	12	3	.9 "
Cane sugar 12%	6	3	3.7 "
Cane sugar 10% + Knop's	12	6	3.3 "
Cane sugar 10% + Knop's − N	13	5	3.7 "
Cane sugar 6%	6	4	6.0 "
Cane sugar 3%	21	13	7.6 "
Cane sugar 3% + Knop's	21	13	11.3 "
Maltose 3%	7	5	4.9 "
Maltose 3% + Knop's	7	4	9.1 "
Dextrin 3%	6	3	11.0 "
Dextrin 3% + Knop's	7	2	12.6 "
Dextrose 3%	18	5	4.8 "
Dextrose 3% + Knop's	18	9	9.8 "
Knop's nutrient	50	3	2.6 "
Knop's nutrient − N	13	0	.4 "
Distilled water	45	8	2.3 "

present in the host upon which the rust as well as the host was able to draw. When reinoculated this was exhausted and there was no infection of hosts in either distilled water or Knop's solution. The results of these experiments indicate that soluble carbohydrates are necessary for the development of the rust.

CARBOHYDRATES SUPPLIED TO PIECES OF LEAF

In the earlier part of this work contaminations occurred which were due to working with imperfectly sterilized leaves and especially with rust spores having saprophytic fungus spores mixed with them.

It was evident that to obtain trustworthy results not only sterile host plants were necessary, but that pure cultures of the rust must also be obtained. This was done as follows:

Pure Cultures of Puccinia Sorghi

The only worker who has given any account of a method to grow rusts in pure culture appears to be Marshall Ward (1902*d*) working with *Puccinia dispersa* upon the bromes. His method consisted in obtaining sterile cultures of the bromes by sterilizing the seed by

"steeping in various antiseptics, or by heating to 60–70° C." The sterile seeds were placed in sterile drying towers, supplied with Knop's mineral solution and aerated with a continuous current of air or were placed in large sterile test tubes which contained the solution. When the first leaf was well developed it was inoculated with uredospores of *Puccinia dispersa*. Good infection was obtained in the inoculated area.

Ward's object in developing this method was to be sure he was working with only one race of *Puccinia dispersa* and not so much to free the rust from saprophytic fungus spores. He does not say that the spores with which he inoculated his sterile plants were free from other fungus spores yet he assumes that he had a pure culture as far as fungi were concerned. He says (p. 459), however, that the method does not exclude harmless bacteria. In order to be sure that spores of saprophytic fungi were not present in the sowing of the rust spores, it would have been necessary to sow the spores of the resulting rust upon nutrient media. This is necessary, since many saprophytic fungus spores do not germinate except in the presence of such nutrient media and so would remain dormant upon the surface of the plant in the infected area and be removed with the spores of the resulting rust.

Two methods have been developed for obtaining pure cultures of the rust. The first method is a modification of Ward's. Large test tubes (30 × 5 cm.) were prepared by filling the lower end with absorbent paper and adding Knop's mineral solution. These were then stoppered with cotton plugs and autoclaved at 10 pounds pressure for 30 minutes. Two or three seeds sterilized in .5 percent $HgCl_2$ solution for 30 minutes were placed in each test tube and the tubes placed in a well lighted window. After the seedlings had developed one or two leaves, they were inoculated with uredospores of *Puccinia Sorghi* (Plate V, figure 1).

The uredospores were obtained from well-developed pustules of *Puccinia Sorghi* and were placed in a capsule of sterile distilled water and thoroughly mixed up. The uredospores of *Puccinia Sorghi*, as well as most other rusts, are much lighter than water and float on the surface, from which they can be removed with a looped platinum wire and placed upon the leaves of the corn. The first trials to obtain infection in this way were failures because of the lack of adhesion of the water drops to the waxy surface of the corn leaves. In ordinary infection work, the leaves are gently drawn between the fingers before

inoculation to remove this waxy substance. Since this method would cause contamination in this case, other means were resorted to. It was noticed that drops of water often condensed upon the leaves during cool nights. Spores were placed in these with resulting infection. As carried out later, the leaves were rubbed with sterile cotton wrapped around sterile forceps and soaked in sterile distilled water. In the drops left adhering to the surface of the leaves, a loop full of uredospores was placed. In this way very good infection was obtained. In most cases, the spores used, after the combined dilution and washing to which they were subjected, were probably sterile, but in order to make sure that there was no contamination, spores from the resulting infection upon these plants were used to inoculate other sterile plants. These spores were taken from the other side of the leaf from that on which the original inoculation had been made. By this means they were obtained from a sterile surface which had not been touched in the original inoculation. The plants of this second series, when once infected, will be reinfected by the spores produced on them as long as they are in good condition, if kept at a temperature favorable for spore germination (Plate V, figure 1).

A second means of obtaining pure cultures of *Puccinia Sorghi* was by means of cut pieces of leaf themselves. Uredospores which had been removed from clean parts of infected plants were diluted in sterile distilled water. Drops from this spore dilution were placed on the surface of pieces of leaf which had been cut from sterile corn plants, and floated upon carbohydrate solutions. A few capsules were contaminated by saprophytic fungi, but more often capsules were obtained which were free from these and the rust produced in such capsules was used to inoculate pieces of leaf in like manner. By inoculating fresh cultures about every two or three weeks, a pure culture of the rust can be kept on hand (Plate V, figure 2).

Experiments 32–41.—These experiments were carried out with pure cultures of both host and rust. Cut pieces of sterile corn leaf were floated upon sterile solutions of 3 and 6 percent cane sugar, 3 percent cane sugar plus Knop's, 3 percent dextrose, 3 percent dextrose plus Knop's, 3 percent maltose, 3 percent maltose plus Knop's, Knop's, and distilled water. After one to three days, uredospores from pure cultures of *Puccinia Sorghi* upon sterile plants were diluted in sterile distilled water and a drop of this spore suspension was placed on each piece of leaf. The capsules were placed in a dark chamber at 20° C.

The results of these experiments are given in the following table.

TABLE VI

Effect of Carbohydrates Supplied to Cut Pieces of Leaf Upon the Development of Puccinia Sorghi

Solution	No. of Pieces of Leaf Used	No. of Pieces Infected	Remarks
Cane sugar 6%	12	6	pustules large
Cane sugar 3%	31	13	" "
Cane sugar 3% + Knop's	36	8	" "
Dextrose 3%	7	3	" "
Dextrose 3% + Knop's	7	4	" "
Maltose 3%	9	5	" "
Maltose 3% + Knop's	10	2	" "
Knop's nutrient	30	2	pustules few, small
Distilled water	30	1	3 pustules, small

Of the pieces of leaf on Knop's nutrient solution which were infected, one piece had one very small pustule, while the other had three very small pustules which were light in color, very unlike the brown color of a vigorous rust. The infection upon carbohydrate solutions varied from a few large brown pustules to a mass of pustules which covered nearly all the area of the piece of leaf (Plate V, figure 2). All the pieces of leaf which were on carbohydrate solutions had the cells of the mesophyll and parenchyma sheaths filled with starch. Pieces of leaf on Knop's solution and distilled water showed no sign of starch. At the end of 14 days, most of the pieces of leaf were alive, as was shown by plasmolyzing with strong KNO_3.

These results agree with those obtained with plants in nutrient solution.

EFFECT OF NUTRITIVE SOLUTIONS UPON SPORE GERMINATION AND CONTINUANCE OF GROWTH

In this work, the uredospores of *Puccinia Sorghi* were sown upon a number of different nutritive solutions. Compounds were used which it was thought would likely be utilized by the rust in its host.

The method employed in the first two experiments was to remove uredospores from an infected plant as carefully as possible and float them on the surface of a sterile solution of the nutrient material to be used. Hanging drops of this spore suspension were then made.

In order to avoid the work necessary for making a large number of van Tieghem cells, the hanging drops were made upon the lid of a

sterile Petri dish. Evaporation from the drops was prevented by either having absorbent paper moistened with the nutrient solution used in the bottom of the dish or by having a small amount of the solution alone. If absorbent paper was used, a V-shaped piece was cut out so that the microscope could be used to observe the development of the rust in the hanging drops, each of which in turn could be brought over the V-shaped opening by turning the cover upon the bottom part of the dish.

The germination could be watched under the 16 and 8 mm. objectives with clearness and the growth and condition of the germ-tubes could be easily followed. Each Petri dish had ten hanging drops on its lid and since three Petri dishes were used for each solution thirty hanging drops were employed for each nutrient medium.

Some contamination resulted in these cultures, but most of the hanging drops showed only a slight growth of saprophytic fungi during the short time that cultures were run.

Experiment 41.—The nutrient media used in this experiment were conductivity water, cane sugar 1 percent, cane sugar 5 percent, maltose 1 percent, maltose 5 percent, leucine 1 percent, asparagine saturated solution, asparagine 1 percent and peptone (Witte's) 1 percent. Besides these, a mineral solution, and carbohydrates plus the mineral solution were used. The mineral solution used was Duggar's standard nutrient solution (1909) for fungi minus the sugar. It consisted of the following, dissolved in 100 cc. of water:

NH_4NO_3	1.00 gm.
KH_2PO_4	.5 gm.
$MgSO_4$	.25 gm.
$FeCl_3$	trace.

The cultures were kept at 17° C. during the experiment. The following table gives the results of the experiment.
Since a dense mass of hyphae was produced during the germination of the spores, the number of germinated spores could not be accurately counted and the amount of germination was estimated by the appearance. In the column under remarks the germ-tube is described whether it produced short side branches or was unbranched. In all these solutions, the rust was dead in about four days.

Experiment 42.—Since the preceding experiment indicated that strong concentrations were injurious to spore germination and that

TABLE VII

The Effect of Nutritive Solutions Upon Spore Germination and Continuance of Growth of Puccinia Sorghi

Solution	Germination	Length of Germ-tube	Remarks
Conductivity water	good	400–500 micr.	unbranched
Mineral nutrient	slight	160 "	"
Cane sugar 5%	fair	160–800 "	"
Cane sugar 1%	good	400–800 "	"
Cane sugar 5% + nut	none		
Maltose 5%	fair	about 100 "	"
Maltose 1%	good	300–800 "	branched some
Maltose 5% + nut	very slight	about 100 "	unbranched
Peptone 1%	fair	" 500 "	branched much
Peptone 1% + nut	none		
Duggar's nut + .5% peptone	none		
Asparagine saturated	slight	" 160 "	branched
Asparagine 1%	fair	80–100 "	"
Leucine 1%	slight	100–400 "	" slightly

they shortened the length of the germ-tube, an experiment was undertaken to study the germination and development of the rust at low concentrations. Two solutions, 1 percent cane sugar and 1 percent cane sugar plus mineral nutrient (see Experiment 41) were used as the basis of this series. Dilutions of 1/2, 1/8, 1/32, 1/128, 1/512, and 1/1024 of this strength were made. Hanging drops of uredospores of *Puccinia Sorghi* were made in these solutions as in the preceding experiment.

No difference was noticed in the amount of germination in these solutions. The length of the germ tube was the greatest (400–800 micr.) in 1/8, 1/2 and 1 percent cane sugar. In the other solutions, the length varied between 160–400 micr. The rust died in all solutions in about three days.

Experiment 43.—In this experiment, plant extracts were used. A decoction of leaves of corn was made by autoclaving pieces of corn plants (1 part by vol. to 5 parts of distilled water) at 10 pounds pressure for 30 minutes. An uncooked extract of the plant was made by cutting up sterile plants as finely as possible and adding sterile distilled water to them and then letting the mixture stand for 24 hours. A third extract was made from sterile germinated seeds which were cut up in sterile distilled water. Two or three seeds were used for 25 cc. of water. Uredospores of *Puccinia Sorghi* from pure cultures of the rust were sown in hanging drops and in capsules of the solutions.

The germination in all cases was good. In the decoction of the

host, the tubes were long (about 800 micr.) and somewhat branched. In the other two solutions, the germ-tubes were long and abundantly branched. Death took place in all these solutions in about four days.

Experiment 44.—Cane sugar 3 percent, cane sugar 3 percent plus Knop's nutrient, dextrose 3 percent, dextrose 3 percent plus Knop's nutrient, Knop's nutrient, and distilled water were used in this experiment. Pieces of corn leaf were floated on these solutions and the solutions were then autoclaved for 30 minutes at 10 pounds pressure. This culture was run at the same time as Experiment 39 and inoculation with uredospores of the rust was made in the same way.

Infection took place upon the living leaves upon the carbohydrate solutions in Experiment 39 in eight days. No development of the rust occurred upon any of the autoclaved leaves.

IV. DISCUSSION

The first question of interest concerns itself with the condition of the tissues in and around the region invaded by the rust. In the development of *Puccinia Sorghi*, it is noticeable that, although most of the cells of the leaf may be invaded by the large haustoria, yet no harmful effect is shown by the host until after some period of time. The rust sends its mycelium through the intercellular spaces and then its haustoria into adjacent cells. The invaded cells retain the characteristics of cells of uninfected tissues. The first sign of effect upon the host is seen in the gradual disappearance of starch from the parenchyma sheaths in the invaded region. Since the parenchyma sheaths serve as a storehouse for the assimilated material from the adjacent region, and since they are not invaded for some time, it would appear that this loss of starch is due, not to a withdrawal of starch from the parenchyma sheath by the fungus itself, but to the utilization by the fungus of the material formed in the neighboring region before it reaches the parenchyma sheaths. That, even at this stage, the rust is not attacking the host vigorously is shown by the development of more or less starch in the parenchyma sheaths of the invaded region depending upon the conditions of photosynthesis at the time of observation. That the rust is having some effect is shown however by the paler color when the parenchyma sheaths of the infected areas are stained with iodine.

This condition prevails up to the time of spore formation. At this time, the rust begins to draw more heavily upon the host in order to

obtain the necessary materials for spore formation. This is evident in the smaller amount of starch present in the parenchyma sheaths in the immediate region of the pustules. Oftentimes the parenchyma sheaths are here entirely devoid of starch, while in the neighboring region starch is present to a considerable extent. Yet even at this time, the cells of the host do not show an injury such as one would expect if the protoplasm itself was attached vigorously by the fungus.

It is only after the number of pustules have increased and spore formation has continued for some time that the host begins to show the effect of the rust's presence. The effect of the rust, even now, is not apparent in the tissues containing the rust, but in the neighboring tissues as is shown by the green color of the infected areas and the lighter green or yellow of the surrounding tissue. The green tissue of the infected areas even at this time, may contain small amounts of starch, but the neighboring dying regions have no indications of starch. It would thus appear that the rust instead of attacking and killing the cells of the tissue in which it is situated has a very different effect upon them. While it is withdrawing food, at the same time it stimulates the infected tissue so that this loss of food is in turn compensated by the withdrawal of food from neighboring uninfected tissue. It would appear that the rust thus destroys the symbiotic balance between the cells of the host and causes some of them to have parasitic relations with the rest. Marshall Ward (1902*b*) and Tubeuf (1897) observed this effect and considered it as evidence of a symbiotic relation between the rusts and their hosts.

As this withdrawal of food goes on the yellowing of the leaf extends farther and farther from the green infected area, the cells of the region gradually die and shrivel up, and the tissue takes on a brown appearance similar to that of cells which have died due to a decomposition of their contents. The infected areas, however, still remain alive for some time, but in these areas death results from two causes. The first of these is the cutting off of the food supply due to the death of the surrounding tissue. This, however, is not probably the principal cause as the green cells of the infected areas could furnish food to prolong their life and that of the rust until by a process of gradual starvation both would die. The principal cause which appears to bring about the death of these areas is the drying up of the leaf as a whole. As Sachs has pointed out the loss of water from dead tissue is much greater than from living. The great evaporation from the

surrounding dead tissue naturally withdraws water from the green infected areas, which have their water supply from the root diminished, and brings about death through drying out.

If conditions at this time are unfavorable for spore germination, such as a low humidity or a too low or too high temperature, the corn plant will be freed from the rust, since the spores formed upon the old leaves will not be able to infect the young newly formed leaves. With the death of the old leaves the host becomes free from the rust. In the same way, oats may become free from their rust.

The work upon the effect of temperature upon the development of the rust also throws some light upon the relation between the rusts and their host. From Experiments 1 and 2 and especially 3 and 4, it is evident that in a saturated atmosphere the development of both *Puccinia coronata* and *Puccinia Sorghi* is retarded by low temperatures.

It is difficult to say just how much these results are due to the direct effect of the temperatures upon the rust, since the rust must be studied in connection with its host. A search of the literature shows that but little work has been done upon the effect of temperature upon the growth of parasitic fungi in their hosts. Sheldon (1902) found that during the winter months the incubation period of *Puccinia Asparagi* was longer than during the summer months when the temperature was higher. Fromme (1913) found a shorter incubation period for *Puccinia coronata* at temperatures between 20° and 30° C. than at lower. Ward (1902*b*) explains the non-infection in some of his experiments by the high temperature, although the host seemed to be unharmed.

The effect of temperature upon the development of saprophytic fungi has received considerable attention. In such experiments, however, the nutrient media remained unchanged. In experiments with the rusts, other conditions besides the temperature alter, since the physiological conditions in the host are altered. It is consequently hard to determine how much of the effect produced on the rust is due directly to the temperature. Lehenbauer (1914) has shown that for corn the optimum temperature for the growth was situated between 29 and 32° C. Sachs (1882) gives 27.2° C. as the optimum temperature for the growth of the root. Besides the effect upon the growth, two of the physiological processes of the host are especially affected. These are respiration and photosynthesis. The respiration of plants increases with the increase in temperature until the injurious effect of

the high temperature brings about a disorganization of the vital functions of the plant. Photosynthesis according to Pfeffer (1900) increases with the temperature up to an optimum, which is approximate to that of growth and then it falls. Matthaei (1905), on the other hand, gives a curve for photosynthesis which resembles that of respiration. This curve, however, is a curve of maximal pnotosynthesis for each temperature. At high temperatures, the maximal photosynthesis is maintained only for a short time. The higher the temperature the sooner the decline sets in and the steeper its slope. The full development of photosynthesis also needs the best conditions of illumination while respiration is not much affected. Consequently in Experiment 4, the food supply available for the fungus at 30° C. is much reduced from that which would be available for it at 20° C., since photosynthesis has fallen off and respiration has increased. At the lower temperatures, although the respiration is lowered, photosynthesis is also reduced and to a much greater degree so that the food available for the fungus is less than at the optimum temperature for photosynthesis.

Although the growth of the fungus is thus influenced to some extent by the effect of temperature upon the amount of food supply available in the host, it is probably the direct effect of the temperature upon the rust itself which is the most important factor in determining the development of the rusts in Experiments 1–4; especially since the temperatures obtained for growth also correspond very well to those for spore germination. The actual temperature of the fungi in these experiments, however, was undoubtedly higher than that recorded, since Matthaei (1905), Ehlers (1915), and others have shown that the internal temperature of leaves is one to ten degrees higher than the surrounding atmosphere, depending upon the amount of illumination, the presence or absence of air currents, and the amount of transpiration.

The evidence concerning the effect of moisture upon the development of the rusts is rather conflicting. Blaringhem (1912, 1913) and Stone and Smith (1899) claim that the rusts are favored by a dry soil. Buchet (1913) believes that a wet soil is favorable. Sirrine (1900) considers that dew is the controlling factor. Smith (1904) finds that a dry atmosphere is unfavorable to *Puccinia Asparagi*, while a dry soil is favorable. From the results of Experiment 5, it is evident that for *Puccinia Sorghi* wet soil and moist atmosphere bring about an increase in vigor, as shown by the greater number of pustules and the

consequently more abundant spores. A humid atmosphere also lessens the transpiration from the dying portions of the leaf and the evaporation from the dead areas so that the infected leaves in a moist atmosphere have a much longer life. In drier air, the infected leaves dry up and the plants become rust free.

The effect of mineral starvation upon the host has been investigated principally by Marshall Ward (1902*c*). Sheldon (1905) and McAlpine (1906) have made some observation upon the effect of soils and manures upon the development of some of the rusts.

Results similar to Marshall Ward's were obtained in Experiments 6 and 7. Infection was obtained upon some plants in all of the mineral nutrients. Table I shows that the best infection was obtained upon plants which were furnished with a full mineral nutrient. A deficiency of an element, however, does not bring about immunity for the host, but it causes a smaller number of plants to be infected and a lessening of the amount of rust as shown by the number of pustules.

That this effect is produced upon the rust is due partly to a lack of these elements for the host and a consequent slow mineral starvation of the rust. This, however, only explains a portion of the effect produced, since the rust can probably obtain these elements in small quantities from the host as long as the host is alive. A portion of the effect produced is at least to be referred to the effect upon the physiology of the host and its greatly reduced ability to manufacture the proper food materials for the rust. That this is true is shown in Table V. In this table it is seen that in solutions in which the host was supplied with a mineral nutrient solution, but was not supplied with carbohydrates and was kept in the dark to prevent their manufacture, the number of plants infected were few. That even this number was infected was due to the fact that the host was not exhausted of soluble carbohydrates, such as the sugars, before infection. The infection of plants supplied with carbohydrates in all cases far outnumber that of the plants without carbohydrates.

The need of carbohydrates is also shown in Experiments 8–21 conducted upon the effect of light upon the development of the rusts. Of the two rusts *Puccinia coronata* upon oats shows the closest relation in this regard. Thus, in Table II, it is shown that the retardation in infection approximates closely the period that the host was left in darkness and consequently the period during which carbohydrates were not being formed. In all cases where the host was kept continuously in darkness after inoculation there was no infection.

That these results are due to the prevention of photosynthesis and not either to non-inoculation due to the negative heliotropic germ-tube of *Puccinia coronata* or to the effect of the lack of light upon the development of the mycelium of the rust is shown in Experiment 11. Since it has been shown that the germ-tubes of the uredospores of *Puccinia coronata* are negatively heliotropic, the explanation of the retardation of the incubation period might be an inability of the germ-tube to enter the stoma while in the dark. This is not the case, as shown by the fact that when plants (C 17.19–C 17.27, Table III) after having been inoculated in the light were placed in the dark for a short period, the incubation period of the rust on them was also lengthened. The demonstration of the presence of mycelium in the leaves of plants inoculated and kept in the dark finally establishes this.

In Experiment 11 there is one case (C 17.35) where the retardation of the incubation period was greater than the time during which the host was in the dark. In this case, the oats were in the dark for eleven days and were consequently so starved and their physiological processes so disarranged that when returned to the light, they carried on their physiological processes poorly and so were able to furnish the rust with only a small amount of food.

The relation of *Puccinia Sorghi* to the carbohydrates formed in the light is not at first glance so striking as in the case of *Puccinia coronata*. Heckel (1912) and Blackshaw (1912) have however shown that corn plants contain from 6 to 9 percent of sugars. Besides this, corn forms starch in the parenchyma sheaths so that under ordinary conditions it contains quite a considerable reserve of carbohydrates. Oats, on the other hand, contain no such reserve and consequently the rust quickly shows the effect when the daily supply is cut off. When means were adopted to decrease the sugar content of corn the results compare more nearly with those obtained with *Puccinia coronata* on oats. It is very evident from Experiments 12–20 that the rust itself does not have any direct relation to light, for infection took place and the rust developed to spore formation in the dark. The same is also shown much better in experiments where carbohydrates were supplied to the host in the dark.

The relation of the rust to the carbohydrate supply is further seen when the host is deprived of its carbon dioxide supply (Experiments 22 and 23). For when the host is prevented from manufacturing carbohydrates by surrounding it with a carbon-dioxide-free atmosphere, there is no development of the rust.

A comparison of the infection of corn when supplied with carbohydrates alone and when supplied with carbohydrates plus Knop's nutrient shows varying results. In spite of the varying results, the experiments clearly show that in all cases there is a much greater infection when carbohydrates are supplied than when there are no carbohydrates present. It is probable that with the slow growth of the host the carbohydrates taken up by the host united with the nitrogen compounds formed in the metabolism of the host and formed proteins for the use of both host and fungus and so the rust did not feel the loss of the mineral elements to a very great extent.

It appears from experiments in which the host was deprived of its soluble carbohydrates that the rust was not able to live upon the host even though the host was alive and consequently did not use the protoplasm of the host, at least directly, as food. Marshall Ward (1904), especially, among the workers upon the rusts has noticed that the protoplasm of the host does not appear to be affected by the rust. In his Croonian lecture (1890), he points out that the relations of the rusts to their hosts are very different from those of a facultative parasite such as *Botrytis*. The rusts he considers as merely tapping the food supply of the host, establishing a relation which approaches symbiosis.

The results obtained agree with Ward's view. The development of the rust upon seedlings or cut leaves of the host furnished with carbohydrates, and the non-development except in a few cases, upon hosts furnished only with distilled water or mineral nutrient indicate that the rust is dependent upon the food supply of the host and does not live upon its protoplasm. The healthy development of host tissue in the infected regions compared with the surrounding dying tissue also shows that not only does the rust not live upon the protoplasm of its host but it even stimulates it to greater activity.

It is possible that the lack of carbohydrates might produce products in the host which are toxic to the rust. Thus amino acids and other products of metabolism might form which in the presence of carbohydrates would unite with them to form proteins again. These products might then inhibit the development of the rust. Experiments 38–40 indicate, however, that such is not the case. In these experiments where cut pieces of leaf were floated upon water and mineral nutrient such products would have had plenty of chance to diffuse out of the leaf. Yet on those solutions deprived of carbohydrates, there was no infection.

Thus the food material for which the rust depends upon the host appears to be the sugars or some of the compounds which they enter into during the formation of proteins, or what is more likely both. Such being the case it should be feasible to grow the rusts saprophytically. So far as I know only two attempts have been made to do this. These have been made by Carleton (1903) who obtained negative results and Ray (1901, 1903) who reports having grown several in culture. As has been pointed out above Ray's results are open to criticism due to the incomplete account of his methods and material.

In the case of *Puccinia Sorghi*, I have not been able to find that various nutrient media had any appreciable effect towards developing a mycelium. Various carbohydrates and organic nitrogenous products with and without mineral salts and at different concentrations showed an effect only upon spore germination and the length of the germ tube (Experiments 41–43). Sterilized pieces of leaf upon solutions such as were used successfully with living pieces of leaf, sterile decoctions of the host and water extracts of the host gave no better results. In all cases, the germ-tube produced by the spore lived only a few days.

Since *Puccinia Sorghi* does not appear to be able to use the sugars directly, but must have them supplied to the host for their development, it appears that it is not the stable carbohydrates or proteins which are to be considered as essential for the metabolism of the rusts. Rather, it is probable that the rust is dependent upon some transitory products in the formation of these substances, as Fromme (1913) has suggested, or it may be that the rust is able to utilize such compounds only in their nascent state, so to speak, when these complex organic compounds are not in a condition of equilibrium. Even among the saprophytic fungi, we have many which prefer certain stereoisomers and it would not be at all surprising to have in the rusts a group of fungi which needs certain isomers for their development. It is in some such explanation very likely that the obligate parasitism of the rusts is to be sought.

V. SUMMARY

1. The optimum temperature for the development of *Puccinia coronata* and *Puccinia Sorghi* is situated at about 20° C. and the maximum for *Puccinia Sorghi* is about 30° C.

2. While *Puccinia Sorghi* is not prevented from developing upon the host under conditions of dry air and soil, moist soil and a humid

atmosphere favor the development of the rust and increase the number of spores formed.

3. *Puccinia coronata* and *Puccinia Sorghi* do not appear to injure the cells of the infected area. The injury produced appears first in the areas surrounding the infected regions. This is probably due to a starvation brought about by a withdrawal of food from them by the infected areas.

4. A starvation of the host of various elements does not bring about immunity from the rust, but reduces the quantity of the rust produced.

5. Light is not necessary for the development of *Puccinia coronata* and *Puccinia Sorghi* when the host is able to obtain a good food supply.

6. When deprived of carbohydrates, light is necessary for the development of *Puccinia coronata* and *Puccinia Sorghi* in that it is necessary for the formation of carbohydrates by the host.

7. When deprived of carbon dioxide, the development of *Puccinia Sorghi* is stopped due to a lack of carbohydrates in the host.

8. Pure cultures of *Puccinia Sorghi* can be maintained upon both sterile seedlings and upon pieces of *Zea Mays* leaf floated upon carbohydrate solutions.

9. *Puccinia Sorghi* develops and forms spores upon seedlings or cut pieces of corn leaf when these are supplied with starch, cane sugar, dextrose, maltose, and dextrin in the dark.

10. When either seedlings or pieces of corn leaf are exhausted of carbohydrates and supplied only with mineral nutrient or water, *Puccinia Sorghi* is not able to develop in the dark.

11. *Puccinia Sorghi* is not able to use maltose, dextrose, cane sugar, asparagine, leucine, peptone with and without mineral salts, or decoctions of the host when supplied to it directly.

12. The obligate parasitism of the rusts is probably explained by their requirement of some transitory or nascent organic products related to the carbohydrates which they obtain in the living host.

VI. LITERATURE CITED

Acton, E. H.

1889. The Assimilation of Carbon by Green Plants from Certain Organic Compounds. Proc. Roy. Soc. London **47**: 150–175.

Balls, W. L.

1905. Infection of Plants by Rust Fungi. New Phytol. **4**: 18, 19.

Bary, A de.

1853. Untersuchungen über die Brandpilze. Berlin.

1863. Recherches sur le développement de quelques champignons parasites. Ann. Sci. Nat. IV. Bot. **20**: 1–148.

1887. Comparative Morphology and Biology of the Fungi, Mycetozoa, and Bacteria. English edition. Oxford.

Blackshaw, G. N.

1912. South Afric. Journ. Sci. 9: 42–48. Abstract in Exp. Sta. Record 30: 14.

Blaringhem, L.

1912. Observations sur la Rouille des Guimauves (Puccinia Malvacearum Mont.). Bull. Soc. Bot. France 59: 765–773.

1913. Sur la transmission héréditaire de la Rouille chez la Rose termiere (Althaea rosea). Compt. Rend. 157: 1536–1538.

Boehm, J.

1883. Ueber Stärkebildung aus Zucker. Bot. Zeit. 41: 32–38, 49–54.

Brefeld, O.

1883. Untersuchungen aus dem Gesammtgebiete Heft 5. Die Brandpilze. Leipzig.

1908. Untersuchungen aus dem Gesammtgebiete Band 14. Die Kultur der Pilze. Münster.

Brown, H. T. and Morris, G. H.

1890. Researches on the Germination of Some of the Gramineae. Journ. Chem. Soc. 57: 458–528.

Buchet, S.

1913. Sur la transmission des rouilles in général et du Puccinia Malvacearum en particulier. Bull. Soc. Bot. France 60: 558–565, 520–524.

Carleton, M. A.

1903. Cultural Methods with Uredineae. Journ. Appl. Micr. Labr. Meth. 6: 2109–2114.

Duggar, B. M.

1909. Fungous Diseases of Plants.

Ehlers, J. H.

1915. The Temperature of Leaves of Pinus in Winter. Amer. Journ. Bot. 2: 32–70.

Eriksson, J. and Henning, E.

1894. Die Hauptresultate einer neuen Untersuchung über die Getreideroste. Zeitschr. Pflanz. 4: 66–73, 140–142, 197–203, 257–262.

Evans, I. B. P.

1907. The Cereal Rusts. I. The Development of their Uredo Mycelia. Annals of Botany 21: 441–466.

Fromme, F. D.

1913. The Culture of Cereal Rusts in the Greenhouse. Bull. Torrey Club 40: 501–521.

1915. Negative Heliotropism of Urediniospore Germ-tubes. Amer. Journ. Bot. 2: 82–85.

Halsted, B. D.

1898. Starch Distribution as Affected by Fungi. Proc. Amer. Assoc. Advanc. Sci. 47: 408, 409.

Heckel, E.

1912. De l'influence de la castration mâle, femelle et totale sur la formation

du sucre dans les tiges du Maïs et du Sorgho sucré. Compt. Rend. 155: 686–690.

Johnson, E. C.

1912. Cardinal Temperatures for the Germination of Uredospores of Cereal Rusts. Abstract in Phytopathology 2: 47.

Jost, Ludwig.

1907. Lectures on Plant Physiology, English edition. Oxford.

Knudson, L.

1916. Influence of Certain Carbohydrates on Green Plants. Cornell Agr. Exp. Sta. Memoir 9.

Laurent, E.

1886. Stärkebildung aus Glycerin. Bot. Zeit. 44: 151.

Laurent, J.

1897. Sur l'absorption des matières organiques par les racines. Compt. Rend. 125: 887–889.

1898. Absorption des hydrates de carbone par les racines. Compt. Rend. 127: 786, 787.

1904. Recherches sur la nutrition carbonée des plantes vertes a l'aide de matières organiques. Rev. Gen. Bot. 16: 14–48, 66–80, 96–128, 155–166, 188–202, 231–241.

Lehenbauer, P. A.

1914. Growth of Maize Seedlings in Relation to Temperature. Physiol. Res. 1: 247–288.

Léveillé, J. H.

1839. Recherches sur le développement des Urédinées. Ann. Sci. Nat. II. Bot. 11: 5–16.

Mains, E. B.

1915. Some Factors Concerned in the Germination of Rust Spores. Mich. Acad. Sci. Rep. 17: 136–140.

Matthaei, Miss G. L. C.

1905. Experimental Researches on Vegetable Assimilation and Respiration. Phil. Trans. London ser. B 197: 47–85.

Mazé, P. and Perrier, A.

1904. Recherches sur l'assimilation de quelques substances ternaires par les végétaux superieurs. Compt. Rend. 139: 470–473.

McAlpine, D.

1906. The Rusts of Australia. Melbourne.

Melhus, I. E.

1912. Culturing of Parasitic Fungi on the Living Host. Phytopathology 2: 197–203.

Plowright, C. B.

1889. A Monograph of the British Uredineae and Ustilagineae. London.

Ray, J.

1901. Cultures et formes atténuées des maladies cryptogamique des végétaux. Compt. Rend. 133: 307–309.

1903. Etude biologique sur le parasitisme: Ustilago Maydis. Compt. Rend. 136: 567–570.

Robinson, W.

1913. On Some Relations between Puccinia Malvacearum and the Tissues of its Host Plant (Althaea rosea). Mem. Proc. Manchester Lit. Phil. Soc. **57**: no. 11.

1914. Some Experiments on the Effect of External Stimuli on the Sporidia of Puccinia Malvacearum (Mont.). Annals of Botany **28**: 330–340.

Sachs, J.

1882. Textbook of Botany. English edition.

Sheldon, J. L.

1902. Preliminary Studies on the Rusts of the Asparagus and Carnation. Science n. ser. **16**: 235–237.

1905. The Effect of Different Soils on the Development of the Carnation Rust. Bot. Gaz. **40**: 225–229.

Sirrine, F. A.

1900. Spraying for Asparagus Rust. N. Y. Agr. Exp. Sta. (Geneva) Bull. 188.

Smith, R. E.

1904. The Water Relation of Puccinia Asparagi. Bot. Gaz. **38**: 19–43.

Stevens, W. C.

1911. Plant Anatomy. Philadelphia.

Stone, G. E. and Smith, R. E.

1899. The Asparagus Rust in Massachusetts. Mass. Agr. Exp. Sta. (Hatch) Bull. 61.

Tieghem, Ph. van.

1873. Recherches Physiologiques sur la Germination. Ann. Sci. Nat. Bot. Series 5, 17: 205–224.

Tischler, G.

1912. Untersuchungen über die Beeinflussung der Euphorbia Cyparissias durch Uromyces Pisi. Flora **104**: 1–64.

Tubeuf, K. F. von.

1897. Diseases of Plants. English edition.

Tulasne, L. R. and Ch.

1847. Memoire sur les Ustilaginées comparées aux Urédinées. Ann. Sci. Nat. III. Bot. **7**: 12–127.

Unger, F.

1834. Die Exantheme der Pflanzen und einige mit diesen verwandte Krankheiten der Gewaechse etc. Review in Ann. Sci. Nat. II. Bot. **2**: 193–215.

Ward, H. M.

1890. Croonian Lecture. On Some Relations between Host and Parasite in Certain Epidemic Diseases of Plants. Proc. Roy. Soc. London **47**: 393–443.

1902a. On the Question of "Predisposition" and "Immunity" in Plants. Proc. Cambridge Phil. Soc. **11**: 307–328.

1902b. On the Relations between Host and Parasite in the Bromes and their Brown Rust, Puccinia dispersa (Erikss.). Annals of Botany **16**: 233–315.

1902c. Experiments on the Effect of Mineral Starvation on the Parasitism of

the Uredine Fungus, Puccinia dispersa, on species of Bromus. Proc. Roy. Soc. London 71: 138–151.

1902d. On Pure Cultures of a Uredine, Puccinia dispersa. Proc. Roy. Soc. London 69: 451–466.

1903. Further Observations on the Brown Rust of the Bromes, Puccinia dispersa (Erikss.) and its adaptive parasitism. Ann. Mycol. 1: 132–151.

1904. On the History of Uredo dispersa Erikss., and the "Mycoplasm" Hypothesis. Phil. Trans. London B196: 29–46.

1905. Recent Researches on the Parasitism of Fungi. Annals of Botany 19: 1–55.

Wilson, J. K.

1915. Calcium Hypochlorite as a Seed Sterilizer. Amer. Journ. Bot. 2: 420–427.

Wuist, Miss E. D.

1913. Sex and Development of the Gametophyte of Onoclea struthiopteris. Physiol. Researches 1: 93–132.

EXPLANATION OF PLATES IV AND V

Plate IV

Fig. 1. Development of *Puccinia Sorghi* in carbon dioxide free atmosphere. *a*. Plant in carbon dioxide free atmosphere (uninfected). *b*. The check (infected).

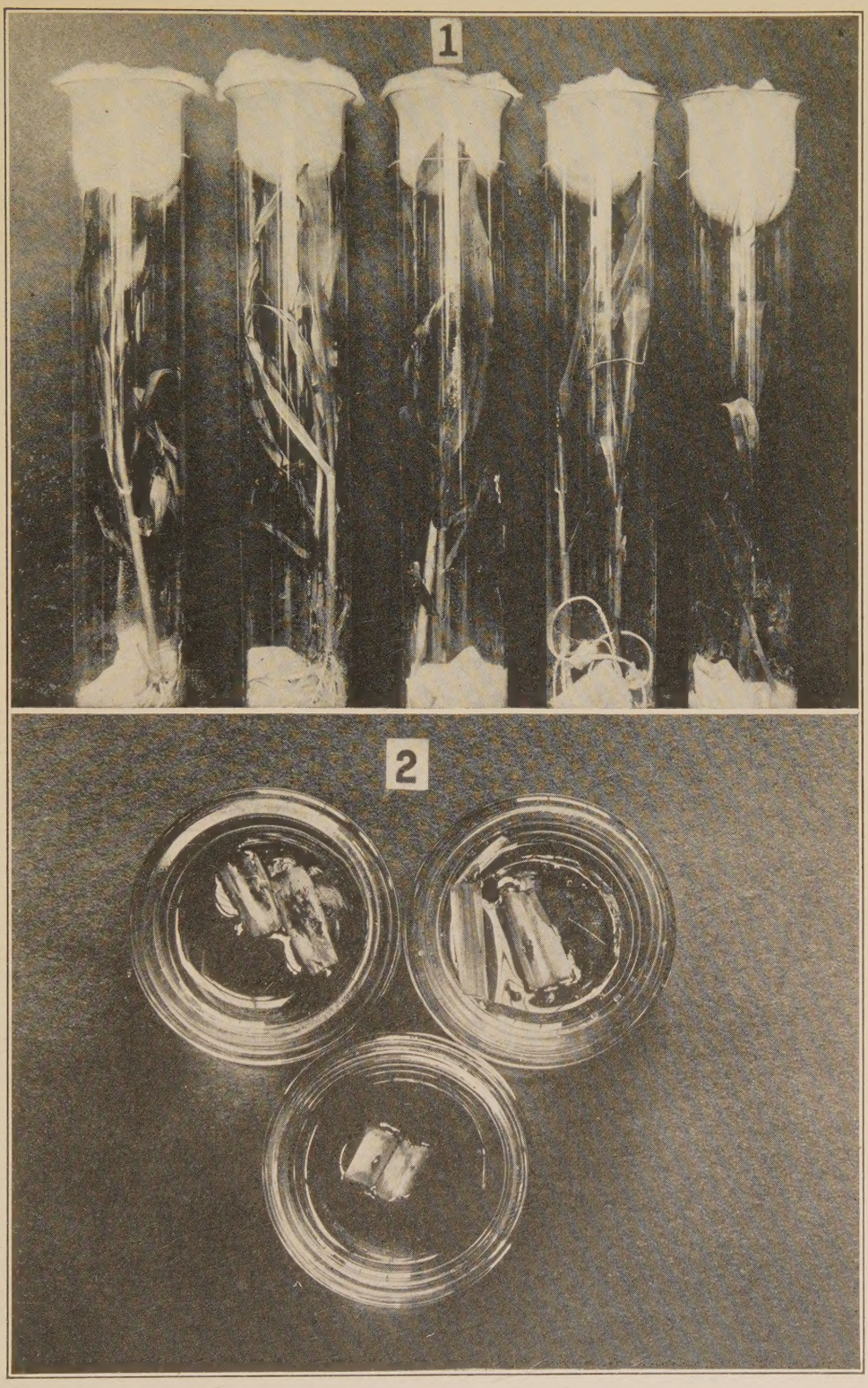
Fig. 2. Development of *Puccinia Sorghi* upon plants supplied with carbohydrates. *a*. Cane sugar 12 percent (infected). *b*. Cane sugar 6 percent (infected). *c*. Cane sugar 3 percent (infected). *d*. Cane sugar 3 percent plus Knop's mineral nutrient (uninfected). *e*. Knop's mineral nutrient (uninfected). *f*. Distilled water (uninfected).

Plate V

Fig. 1. Pure culture of *Puccinia Sorghi* and its host.

Fig. 2. Pure culture of *Puccinia Sorghi* upon cut pieces of corn leaf floated upon carbohydrate solution (looking down upon the capsules containing the leaves).

Mains: Relation of Rusts to Physiology of their Hosts



MAINS: RELATION OF RUSTS TO PHYSIOLOGY OF THEIR HOSTS